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PTO/SB/16 (10-01)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No.

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☒ *Additional inventors are being named on the 1 separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

METHODS FOR DETECTING AND TREATING CANCER

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☒ Application Data Sheet. See 37 CFR 1.76

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☒ Applicant claims small entity status. See 37 CFR 1.27.

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☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____.

Respectfully submitted,

SIGNATURE M. Gravelle Date JUNE 6, 2003

TYPED or PRINTED NAME MICHELINE GRAVELLE REGISTRATION NO. *(if appropriate)* 40,261

TELEPHONE 416-364-7311 Docket Number: 7685-55

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Additional Page

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Docket Number	7685-55

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Number 2 of 2

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PTO/SB/17 (01-03)

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☑ Applicant claims small entity status. See 37 CFR 1.27

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First Named Inventor	KELLY M. McNAGNY
Examiner Name	
Art Unit	
Attorney Docket No.	7685-55

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below	Fee Paid
Total Claims	- 20** =		X		= 0
Independent Claims	- 3** =		X		= 0
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
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Patent Application Data Sheet

Application Information

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Initial 6/6/03

B&P File No. 7685-55

BERESKIN & PARR

UNITED STATES PROVISIONAL

Title: METHODS FOR DETECTING AND TREATING CANCER

Inventors: Kelly M. McNagny, Calvin Roskelley, Aruna Somasiri and David Huntsman

B&P File No. 7685-55

Title: METHODS FOR DETECTING AND TREATING CANCER

FIELD OF THE INVENTION

The present invention relates to methods and kits for detecting and monitoring the progression of cancer, in particular breast cancer. The invention also includes methods of treating cancer.

BACKGROUND OF THE INVENTION

Metastatic breast cancer is the leading cause of death among women between the ages of 15 and 54 and affects approximately 13% of women during their lifespan. These can be grossly categorized as ductal or lobular depending on their site of origin in normal breast tissue. Tumors usually begin as non-invasive cells at the site of tumor origin, spread to surrounding tissue in the breast and eventually become fully metastatic and migrate to the lymph nodes and other parts of the body.

There is increasing evidence that cell-cell adhesion is a potent suppressor of metastatic breast cancer progression (Berx and Van Roy, 2001). For example, in infiltrating lobular breast carcinomas E-cadherin is often lost and the resulting disruption of adherens junctions initiates a complete dissolution of cell-cell adhesion which allows single cells to break away from the primary tumor and invade the stroma in a single file pattern (Cleton Jansen et al., 2002). Alterations in cell adhesion are more subtle in infiltrating ductal carcinomas where invasion is characterized by the movement of clusters of cells into the stroma (Page and Simpson, 2000). In the latter situation adherens junctions are often present (Acs et al., 2001; Gillett et al., 2001) but there appears to be a general loss of polarity that is characterized by the mislocalization of apical markers such as MUC-1 (McGuckin et al., 1995; Mommers et al., 1999; Diaz et al., 2001; Rahn et al., 2001) that may be fueled by the disruption of tight junctions (Hoover et al., 1997; Kramer et al., 2000; Kominsky et al., 2003). While transcriptional repressors of E-cadherin expression have been identified (Batle et al., 2000; Cano et al., Guaita et al., 2001), little is known about the mechanism

responsible for the disruption of tight junctions during breast tumor progression.

Podocalyxin, (also called podocalyxin-like protein 1 (PCLP-1), Myb-Ets-transformed progenitor (MEP21) or thrombomucin) is a heavily sialyated and sulfated integral membrane glycoprotein that interacts with the actin cytoskeleton. It belongs to the CD34 family of sialomucins and is highly expressed on the surface of hematopoeitic progenitors, vascular endothelia and podocytes which form a tight junction-free epithelial meshwork that surrounds glomerular capillaries in the kidney (Kerjaschki et al., 1984; Kershaw et al., 1995; McNagny et al., 1997). Mounting evidence suggest that the primary function of this molecule is to act as a type of molecular "Teflon™" to block inappropriate cell adhesion. For example, as kidney podocytes begin to express podocalyxin they undergo a dramatic morphological shift from adherent, tight junction-associated monolayers surrounding the glomerular capillaries to a more modified cell layer lacking tight junctions and with extensive fully-interdigitated foot processes that are separated from each other by slit diaphragms. These podocalyxin-covered slit diaphragms form the primary filtration apparatus of the kidney. Deletion of the podocalyxin-encoding gene in mice results in the persistence of tight-junctions between podocytes, a lack of foot process formation and perinatal death due to anuria and high blood pressure (Doyonnas et al., 2001). Conversely, when podocalyxin is ectopically expressed in kidney epithelial cell monolayers, tight junctions and adherens junctions are both disrupted (Takeda et al., 2000). Thus, both gain-of-function and loss-of-function experiments suggest that podocalyxin acts as a tissue-specific anti-adhesin during normal kidney development (Takeda et al., 2001, Doyonnas et al., 2001).

SUMMARY OF THE INVENTION

The inventors have shown that podocalyxin is a prognostic indicator of tumor metastasis and that it plays an active role in making cells less adherent and more invasive.

Accordingly, in one embodiment, the present invention provides a method for detecting cancer in a patient comprising:

(a) obtaining a sample from the patient;

(b) detecting the level of podocalyxin in the sample; and

(c) comparing the level of podocalyxin in the sample to a control sample, wherein increased levels of podocalyxin as compared to the control indicates that the patient has cancer.

In another embodiment, the present invention provides a method for monitoring the progression of cancer in a patient, comprising:

(a) obtaining a sample from a patient;

(b) determining the level of podocalyxin in the sample;

(c) repeating steps (a) and (b) at a later point in time and comparing the result of step (b) with the result of step (c) wherein a difference in the level of podocalyxin expression is indicative of the progression of the cancer in the patient.

In a further embodiment, the present invention provides a method for determining whether or not a cancer is metastatic in a patient comprising:

(a) obtaining a sample from the patient;

(b) detecting the level of podocalyxin in the sample; and

(c) comparing the level of podocalyxin in the sample to a control sample, wherein increased levels of podocalyxin as compared to the control indicates that the cancer is metastatic.

In preferred embodiments of the invention, the above methods are used to detect breast cancer.

The present invention includes methods of treating cancer by modulating, preferably inhibiting, the levels of podocalyxin on the cancer. The application also includes methods for the identification of compounds that modulate the biological activity of podocalyxin that may be used for the treatment of cancers with increased expression of podocalyxin.

Accordingly, the present invention provides a method of modulating cancer cell growth by administering an effective amount of an agent that modulates podocalyxin to a cell or animal in need thereof.

The present invention also includes screening assays for identifying agents that modulate podocalyxin and that are useful in modulating cancer cell growth. Agents that modulate include agents that stimulate podocalyxin (podocalyxin agonists) and agents that inhibit podocalyxin (podocalyxin antagonists).

Accordingly, the present invention provides a method for identifying a compound that modulates podocalyxin comprising:

(a) incubating a test compound with podocalyxin or a nucleic acid encoding podocalyxin; and

(b) determining the effect of the compound on podocalyxin activity or expression and comparing with a control (i.e. in the absence of the test substance), wherein a change in the podocalyxin activity or expression as compared to the control indicates that the test compound modulates podocalyxin.

The present invention includes pharmaceutical compositions containing one or more modulators of podocalyxin. Accordingly, the present invention provides a pharmaceutical composition for use in modulating cancer cell growth comprising an effective amount of podocalyxin modulator in admixture with a suitable diluent or carrier.

In one embodiment, the present invention provides a pharmaceutical composition for use in treating cancer comprising an effective amount of a podocalyxin antagonist in admixture with a suitable diluent or carrier.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 shows podocalyxin immunostaining of normal tissues and the tumor microarray. In positive control kidney tissue (A) the podocytes within the glomerulus were highly positive while the tubular epithelium was negative (see inset). The vascular endothelium of the glomerulus and within the kidney cortex was also positive. In normal breast tissue (B) positive staining was observed in the vascular endothelium (arrows) and the apical regions of luminal breast epithelial cells (see inset; arrowheads). On the tissue microarray invasive breast carcinomas were scored as: '0' (ie. C) if there was no discernable staining on the carcinoma cells (see inset; positive staining is on endothelial cells); '1' (ie. D) if less than 10% of the cells stainined positively; '2' (ie. E) if there was diffuse staining in more than 10% of the cells and/or strong cytoplasmic staining in less than 50% of the cells; or '3' if there was strong cytoplasmic staining in more than 50% of the cells (ie. F).

Figure 2 consists of two graphs illustrating the prognostic significance of podocalyxin expression in breast tumors (Kaplan-Meier survival analysis). Disease specific survival at all expression levels indicates that only the high podocalyxin expression level (+3) is prognostically significant (A). Therefore, expression levels 0 to 2 were combined as "no or low podocalyxin" and +3 as was designated as "high podocalyxin" (B).

Figure 3 illustrates the functional significance of podocalyxin overexpression in MCF-7 breast carcinoma cells.

Figure 3A shows the endogenous levels in three human breast carcinoma lines as assessed by Western blotting with an antibody specific for human podocalyxin. Note that podocalyxin was modestly expressed in well-behaved T47D and MCF-7 cells compared to the highly invasive MDA231 cell line.

Figure 3B is a series of photographs showing MCF-7 cells that were control transfected or stably transfected with an expression vector containing both GFP and mouse podocalyxin. Control transfected MCF-7 cells formed classical cobblestone epithelial monolayers (top panel) while

bulging cells were shed from the surface of the GFP/Podocalyxin transfected cells (middle panel). GFP (green) and mouse podocalyxin (red) were coordinately expressed in a heterogenous manner (lower panel). (upper two panels live phase microscopy, bar = 50μm; lower panel, Z-series confocal dual fluorescence microscopy for GFP and mouse-specific podocalyxin immunostaining; bar = 15μm).

Figure 3C is a series of photographs of transfected MCF-7 cells that were triple stained for mouse podocalyxin (red), DNA/Nuclei (blue) and either the adherens junction protein E-cadherin or the tight junction protein occludin (green). Note that where podocalyxin was not expressed E-cadherin was localized basolaterally and occludin was localized at apical terminal bars. In contrast, where podocalyxin was expressed the cells bulged apically (note upward movement of blue nuclei) and both E-cadherin and occludin localization became depolarized (Z-series confocal microscopy, bar=15μm)

DETAILED DESCRIPTION OF THE INVENTION

I. Diagnostic Methods

The present inventors have determined that podocalyxin is a functionally important molecule in tumor progression. Using a tissue microrray (TMA), the inventors assessed podocalyxin expression and localization in a series of 270 invasive human breast carcinomas for which full clinicopathologic follow up and outcome was present (Gilks et al., 2003). Podocalyxin was found to be highly expressed and diffusely distributed in a small subset of these tumors. It was also found that high podocalyxin expression was a clear and independent prognostic indicator of poor outcome in this tumor subset. To test the functional consequences of this increased expression, murine podocalyxin was ectopically expressed in human MCF-7 breast carcinoma cells that normally grow as adherent monolayers with abundant adherens junctions and tight junctions. Low level ectopic podocalyxin expression lead to the disruption of both adherens and tight junctions while high cells expressing high levels of the protein were de-polarized and actively extruded from otherwise cohesive MCF-7 monolayers. The data demonstrates that podocalyxin is a prognostic indicator of tumor

metastasis and that it plays an active role in making cells less adherent and more invasive. The inventors have also shown that podocalyxin is involved in decreasing the apical/basal cell polarity of breast tissues, a hallmark of solid tumor progression. The inventors have also shown that podocalyxin expression is dramatically increased during hypoxia, as the rapid proliferation of cells during tumor progression causes the tissue to become hypoxic. Therefore, podocalyxin is a marker of solid tumor progression and a marker of tumor hypoxia.

Accordingly, evaluating podocalyxin levels may be used in the prognostic and diagnostic evaluation of cancers involving podocalyxin, the identification of subjects with a predisposition to such cancers, and in the monitoring of the progress of patients with podocalyxin related cancers.

In an embodiment of the invention, a method is provided for detecting cancer in a patient comprising:

(a) obtaining a sample from the patient;

(b) detecting the level of podocalyxin in the sample; and

(c) comparing the level of podocalyxin in the sample to a control sample, wherein increased levels of podocalyxin as compared to the control indicates that the patient has cancer.

The term "podocalyxin" as used herein is synonymous with podocalyxin-like protein 1 (PCLP-1), Myb-Ets-transformed progenitor (MEP21) or thrombomucin and is a heavily sialyated and sulfated integral membrane glycoprotein that interacts with the actin cytoskeleton. The term podocalyxin includes all of the known podocalyxin molecules including those deposited in GenBank under accession number U97519 or those referred to in Kershaw et al. (Kershaw DB, Beck SG, Wharram BL, Wiggins JE, Goyal M, Thomas PE, Wiggins RC., Molecular cloning and characterization of human podocalyxin-like protein. Orthologous relationship to rabbit PCLP1 and rat podocalyxin. J Biol Chem. 1997 Jun 20;272(25):15708-14) as well as any isoforms, variants, analogs, derivatives or fragments thereof that are useful in detecting cancer.

The phrase "detecting the level of podocalyxin" includes the detection of the levels of the podocalyxin protein as well as detection of the levels of nucleic acid molecules encoding the podocalyxin protein. Methods for detecting proteins and nucleic acids are discussed in greater detail below.

Podocalyxin is alternatively spliced to give two isoforms of the protein core; one with a long cytoplasmic tail and one with a short cytoplasmic tail. Data suggests that the short form may be a better blocker of adhesion. Consequently, in a specific embodiment, the methods of the invention are used to detect the short form of podocalyxin.

The term "cancer" as used herein includes all cancers that are associated with increased expression of podocalyxin. In a preferred embodiment, the cancer is breast cancer, more preferably invasive breast carcinoma.

The term "sample from a patient" as used herein means any sample containing cancer cells that one wishes to detect including, but not limited to, biological fluids, tissue extracts, freshly harvested cells, and lysates of cells which have been incubated in cell cultures. In a preferred embodiment, the sample is breast tissue.

The term "control sample" includes any sample that can be used to establish a base or normal level, and may include tissue samples taken from healthy persons or samples mimicking physiological fluid.

The method of the invention may be used in the diagnosis and staging of cancer, in particular breast cancer. The invention may also be used to monitor the progression of a cancer and to monitor whether a particular treatment is effective or not. In particular, the method can be used to confirm the absence or removal of all tumor tissue following surgery, cancer chemotherapy, and/or radiation therapy. The methods can further be used to monitor cancer chemotherapy and tumor reappearance.

In an embodiment, the invention contemplates a method for monitoring the progression of cancer in a patient, comprising:

(a) obtaining a sample from a patient;

(b) determining the level of podocalyxin expression in the sample;

(c) repeating steps (a) and (b) at a later point in time and comparing the result of step (b) with the result of step (c) wherein a difference in the level of podocalyxin expression is indicative of the progression of the cancer in the patient.

In particular, increased levels of podocalyxin at the later time point may indicate that the cancer is progressing and that the treatment (if applicable) is not being effective. In contrast, decreased levels of podocalyxin at the later time point may indicate that the cancer is regressing and that the treatment (if applicable) is effective.

The inventors have also shown that podocalyxin is a marker of tumor metastasis. Accordingly, the present invention provides a method of determining whether or not a cancer is metastatic in a patient comprising:

(a) obtaining a sample from the patient;

(b) detecting the level of podocalyxin in the sample; and

(c) comparing the level of podocalyxin in the sample to a control sample, wherein increased levels of podocalyxin as compared to the control indicates that the cancer is metastatic.

A variety of methods can be employed for the above described diagnostic and prognostic evaluation of cancers involving podocalyxin, and the identification of subjects with a predisposition to such disorders. Such methods may rely, for example, the detection of nucleic acid molecules encoding podocalyxin, and fragments thereof, or the detection of the podocalyxin protein using, for example, antibodies directed against podocalyxin, including peptide fragments. Each of these is described below.

(a) Methods for Detecting Nucleic Acid Molecules

In one embodiment, the methods of the invention involve the detection of nucleic acid molecules encoding podocalyxin. Those skilled in the art can construct nucleotide probes for use in the detection of nucleic acid sequences encoding podocalyxin in samples. Suitable probes include nucleic acid molecules based on nucleic acid sequences encoding at least 5

sequential amino acids from regions of podocalyxin, preferably they comprise 15 to 30 nucleotides. A nucleotide probe may be labeled with a detectable substance such as a radioactive label which provides for an adequate signal and has sufficient half-life such as ^{32}P, ^{3}H, ^{14}C or the like. Other detectable substances which may be used include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes, antibodies specific for a labeled antigen, and luminescent compounds. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleotide to be detected and the amount of nucleotide available for hybridization. Labeled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook et al, 1989, Molecular Cloning, A Laboratory Manual (2nd ed.). The nucleic acid probes may be used to detect genes, preferably in human cells, that encode podocalyxin. The nucleotide probes may also be useful in the diagnosis of disorders involving a podocalyxin in monitoring the progression of such disorders; or monitoring a therapeutic treatment. In an embodiment, the probes are used in the diagnosis of, and in monitoring the progression of cancer, preferably breast cancer.

The probe may be used in hybridization techniques to detect genes that encode podocalyxin proteins. The technique generally involves contacting and incubating nucleic acids (e.g. recombinant DNA molecules, cloned genes) obtained from a sample from a patient or other cellular source with a probe under conditions favorable for the specific annealing of the probes to complementary sequences in the nucleic acids. After incubation, the non-annealed nucleic acids are removed, and the presence of nucleic acids that have hybridized to the probe if any are detected.

The detection of nucleic acid molecules may involve the amplification of specific gene sequences using an amplification method such as polymerase chain reaction (PCR), followed by the analysis of the amplified molecules using techniques known to those skilled in the art. Suitable primers can be routinely designed by one of skill in the art.

Hybridization and amplification techniques described herein may

be used to assay qualitative and quantitative aspects of expression of genes encoding podocalyxin. For example, RNA may be isolated from a cell type or tissue known to express a gene encoding podocalyxin, and tested utilizing the hybridization (e.g. standard Northern analyses) or PCR techniques referred to herein. The techniques may be used to detect differences in transcript size which may be due to normal or abnormal alternative splicing. The techniques may be used to detect quantitative differences between levels of full length and/or alternatively splice transcripts detected in normal individuals relative to those individuals exhibiting symptoms of a cancer involving a podocalyxin protein or gene.

The primers and probes may be used in the above described methods *in situ* i.e. directly on tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections.

Accordingly, the present invention provides a method of detecting cancer in a patient comprising:

(a) obtaining a sample from the patient;

(b) extracting nucleic acid molecules comprising the podocalyxin gene or portion thereof from the sample;

(c) amplifying the extracted nucleic acid molecules using the polymerase chain reaction;

(d) determining the presence of nucleic acid molecules encoding podocalyxin; and

(e) comparing the level of podocalyxin in the sample to a control sample, wherein increased levels of podocalyxin as compared to the control indicates that the patient has cancer.

(b) Methods for Detecting Podocalyxin Proteins

In another embodiment, the methods of the invention involve the detection of the podocalyxin protein. In one embodiment, the podocalyxin protein is detected using antibodies that specifically bind to podocalyxin. Antibodies to podocalyxin are known in the art as described in Example 1.

Antibodies to the podocalyxin may also be prepared using techniques known in the art. For example, by using a peptide of podocalyxin,

polyclonal antisera or monoclonal antibodies can be made using standard methods. A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the peptide which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the protein or peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera.

To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g., the hybridoma technique originally developed by Kohler and Milstein (Nature 256, 495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., Immunol. Today 4, 72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. Monoclonal Antibodies in Cancer Therapy (1985) Allen R. Bliss, Inc., pages 77-96), and screening of combinatorial antibody libraries (Huse et al., Science 246, 1275 (1989)). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and the monoclonal antibodies can be isolated.

The term "antibody" as used herein is intended to include fragments thereof which also specifically react with a podocalyxin or fragments thereof. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above. For example, F(ab')2 fragments can be generated by treating antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

Chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region are also contemplated within the scope of the invention. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. Conventional methods may be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes the gene product of podocalyxin antigens of the invention (See, for example, Morrison et al., Proc. Natl Acad. Sci. U.S.A. 81,6851 (1985); Takeda et al., Nature 314, 452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom patent GB 2177096B). It is expected that chimeric antibodies would be less immunogenic in a human subject than the corresponding non-chimeric antibody.

Monoclonal or chimeric antibodies specifically reactive with a protein of the invention as described herein can be further humanized by producing human constant region chimeras, in which parts of the variable regions, particularly the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such immunoglobulin molecules may be made by techniques known in the art, (e.g., Teng et al., Proc. Natl. Acad. Sci. U.S.A., 80, 7308-7312 (1983); Kozbor et al., Immunology Today, 4, 7279 (1983); Olsson et al., Meth. Enzymol., 92, 3-16 (1982)), and PCT Publication WO92/06193 or EP 0239400). Humanized antibodies can also be commercially produced (Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.)

Specific antibodies, or antibody fragments, such as, but not limited to, single-chain Fv monoclonal antibodies reactive against podocalyxin may also be generated by screening expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with peptides produced from the nucleic acid molecules of podocalyxin. For example, complete Fab fragments, VH regions and FV regions can be

expressed in bacteria using phage expression libraries (See for example Ward et al., Nature 341, 544-546: (1989); Huse et al., Science 246, 1275-1281 (1989); and McCafferty et al. Nature 348, 552-554 (1990)). Alternatively, a SCID-hu mouse, for example the model developed by Genpharm, can be used to produce antibodies or fragments thereof.

Antibodies specifically reactive with podocalyxin, or derivatives, such as enzyme conjugates or labeled derivatives, may be used to detect podocalyxin in various samples (e.g. biological materials). They may be used as diagnostic or prognostic reagents and they may be used to detect abnormalities in the level of protein expression, or abnormalities in the structure, and/or temporal, tissue, cellular, or subcellular location of a podocalyxin. *In vitro* immunoassays may also be used to assess or monitor the efficacy of particular therapies. The antibodies of the invention may also be used *in vitro* to determine the level of expression of a gene encoding podocalyxin in cells genetically engineered to produce a podocalyxin protein.

The antibodies may be used in any known immunoassays which rely on the binding interaction between an antigenic determinant of podocalyxin and the antibodies. Examples of such assays are radioimmunoassays, enzyme immunoassays (e.g. ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination, and histochemical tests. The antibodies may be used to detect and quantify podocalyxin in a sample in order to determine its role in cancer and to diagnose the cancer.

In particular, the antibodies of the invention may be used in immuno-histochemical analyses, for example, at the cellular and sub-subcellular level, to detect a podocalyxin protein, to localize it to particular cells and tissues, and to specific subcellular locations, and to quantitate the level of expression.

Cytochemical techniques known in the art for localizing antigens using light and electron microscopy may be used to detect podocalyxin. Generally, an antibody of the invention may be labeled with a detectable substance and a podocalyxin protein may be localised in tissues and cells

based upon the presence of the detectable substance. Examples of detectable substances include, but are not limited to, the following: radioisotopes (e.g., ^{3}H, ^{14}C, ^{35}S, ^{125}I, ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), luminescent labels such as luminol; enzymatic labels (e.g., horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase, acetylcholinesterase), biotinyl groups (which can be detected by marked avidin e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods), predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached via spacer arms of various lengths to reduce potential steric hindrance. Antibodies may also be coupled to electron dense substances, such as ferritin or colloidal gold, which are readily visualised by electron microscopy.

The antibody or sample may be immobilized on a carrier or solid support which is capable of immobilizing cells, antibodies etc. For example, the carrier or support may be nitrocellulose, or glass, polyacrylamides, gabbros, and magnetite. The support material may have any possible configuration including spherical (e.g. bead), cylindrical (e.g. inside surface of a test tube or well, or the external surface of a rod), or flat (e.g. sheet, test strip). Indirect methods may also be employed in which the primary antigen-antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against podocalyxin protein. By way of example, if the antibody having specificity against podocalyxin protein is a rabbit IgG antibody, the second antibody may be goat anti-rabbit gamma-globulin labeled with a detectable substance as described herein.

Where a radioactive label is used as a detectable substance, podocalyxin may be localized by radioautography. The results of radioautography may be quantitated by determining the density of particles in the radioautographs by various optical methods, or by counting the grains.

Labeled antibodies against podocalyxin protein may be used in locating tumor tissue in patients undergoing surgery i.e. in imaging. Typically for *in vivo* applications, antibodies are labeled with radioactive labels (e.g. iodine-123, iodine-125, iodine-131, gallium-67, technetium-99, and indium-111). Labeled antibody preparations may be administered to a patient intravenously in an appropriate carrier at a time several hours to four days before the tissue is imaged. During this period unbound fractions are cleared from the patient and the only remaining antibodies are those associated with tumor tissue. The presence of the isotope is detected using a suitable gamma camera. The labeled tissue can be correlated with known markers on the patient's body to pinpoint the location of the tumor for the surgeon.

Accordingly, in another embodiment the present invention provides a method for detecting cancer in a patient comprising:

(a) obtaining a sample from the patient;

(b) contacting the sample with an antibody that binds to podocalyxin;

(c) detecting the level of podocalyxin in the sample; and

(d) comparing the level of podocalyxin in the sample to a control sample, wherein increased levels of podocalyxin as compared to the control indicates that the patient has cancer.

In a specific embodiment of the invention, breast tissue samples can be screened using an anti-podocalyxin antibody, such as mouse monoclonal anti-human podocalyxin antibody 3D3. Antibody binding is detected using an appropriate detection system, preferably the Envision detection system, and staining is scored based on the intensity of cellular staining and the proportion of cells stained. Tissue samples are designated "0" (no discernable podocalyxin staining), "1" (weak staining in the majority of tumor cells), "2" (a mixture of weak and intense membrane staining) or "3" (strong staining in the majority of tumor cells). Tissue samples exhibiting strong staining in the majority of tumor cells (designated "3") have a significantly poorer outcome when compared with the other three designations.

II. Kits

The methods described herein may be performed by utilizing pre-packaged diagnostic kits comprising the necessary reagents to perform any of the methods of the invention. For example, the kits may include at least one specific nucleic acid or antibody described herein, which may be conveniently used, e.g., in clinical settings, to screen and diagnose patients and to screen and identify those individuals exhibiting a predisposition to developing cancer. The kits may also include nucleic acid primers for amplifying nucleic acids encoding podocalyxin in the polymerase chain reaction. The kits can also include nucleotides, enzymes and buffers useful in the method of the invention as well as electrophorectic markers such as a 200 bp ladder. The kit will also include detailed instructions for carrying out the methods of the invention.

III. Therapeutic Methods

The finding by the present inventors that podocalyxin is involved in tumor progression allows the development of therapies to treat cancer including the identification of compounds that modulate podocalyxin. The present invention includes methods of treating cancer by modulating, preferably inhibiting, the levels of podocalyxin on the cancer. The application also includes methods for the identification of compounds that modulate the biological activity of podocalyxin that may be used for the treatment of cancers with increased expression of podocalyxin.

Accordingly, the present invention provides a method of modulating cancer cell growth by administering an effective amount of an agent that modulates podocalyxin to a cell or animal in need thereof.

The terms "podocalyxin" and "cancer" as used herein are as defined above in Section I.

The phrase "agent that modulates podocalyxin" includes any agent that can stimulate or activate podocalyxin (i.e. podocalyxin agonists) as well as any agent that can inhibit or suppress podocalyxin (i.e. podocalyxin antagonists). Specific examples of podocalyxin modulators are given below.

The phrase "modulate cancer cell growth" as used herein refers to the inhibition or suppression as well as the activation or stimulation of the formation, differentiation, growth or development of cancer cells.

The phrase "effective amount" as used herein means an amount effective, at dosages and for periods of time necessary to achieve the desired results (e.g. the modulation of cancer cell growth). Effective amounts of a molecule may vary according to factors such as the disease state, age, sex, weight of the animal. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The term "animal" as used herein includes all members of the animal kingdom which express podocalyxin, preferably humans.

The term "a cell" includes a single cell as well as a plurality or population of cells. Administering an agent to a cell includes both *in vitro* and *in vivo* administrations.

In one aspect, the present invention provides a method of inhibiting cancer cell growth or treating cancer comprising administering an effective amount of podocalyxin antagonist to a cell or animal in need thereof.

The phrase "inhibiting cancer cell growth" means that the growth of the cancer cell is decreased or reduced as compared to the growth of the cancer cell in the absence of the podocalyxin antagonist.

The term "treatment or treating" as used herein means an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treating" can also mean prolonging survival as compared to expected survival if not receiving treatment.

In a preferred embodiment, the therapeutic methods of the invention are used to treat breast cancer.

The phrase "podocalyxin antagonist" means any agent that can inhibit or reduce the activity, function or levels of expression of podocalyxin on a cancer cell. Examples of podocalyxin antagonists include, but are not limited to, an antibody, small molecule, peptide mimetic, an antisense oligonucleotide to podocalyxin or any molecule or protein that can crosslink podocalyxin on the surface of the tumor cell.

In one embodiment, the podocalyxin antagonist is an antibody that binds to podocalyxin. The preparation of antibodies to podocalyxin are described above in Section I and the same procedures can be used to prepare antibodies with therapeutic efficacy. In a preferred embodiment, the antibody will selectively bind a tumor specific isoform of podocalyxin but not the isoform found on normal cells. Accordingly, the present invention provides a method of treating cancer comprising administering an effective amount of an antibody that can bind podocalyxin to a cell or animal in need thereof. Coating cancer cells with anti-podocalyxin antibodies may inhibit cell growth or induce apoptosis. In specific embodiments the antibody can be coupled to a toxin that can cause the death of the cancer cell.

In another embodiment, the podocalyxin antagonist is an antisense oligonucleotides that can modulate the expression and/or activity of podocalyxin on cancer cells.

The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complimentary to its target.

The term "oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and intersugar (backbone) linkages. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted oligonucleotides may be preferred over naturally occurring forms because of properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes chimeric oligonucleotides

which contain two or more chemically distinct regions. For example, chimeric oligonucleotides may contain at least one region of modified nucleotides that confer beneficial properties (e.g. increased nuclease resistance, increased uptake into cells), or two or more oligonucleotides of the invention may be joined to form a chimeric oligonucleotide.

The antisense oligonucleotides of the present invention may be ribonucleic or deoxyribonucleic acids and may contain naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The oligonucleotides may also contain modified bases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

Other antisense oligonucleotides of the invention may contain modified phosphorous, oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. For example, the antisense oligonucleotides may contain phosphorothioates, phosphotriesters, methyl phosphonates, and phosphorodithioates. In an embodiment of the invention there are phosphorothioate bonds links between the four to six 3'-terminus bases. In another embodiment phosphorothioate bonds link all the nucleotides.

The antisense oligonucleotides of the invention may also comprise nucleotide analogs that may be better suited as therapeutic or experimental reagents. An example of an oligonucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in the DNA (or RNA), is replaced with a polyamide backbone which is similar to that found in peptides (P.E. Nielsen, et al Science 1991, 254, 1497). PNA analogues have been shown to be resistant to degradation by

enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind stronger to a complimentary DNA sequence due to the lack of charge repulsion between the PNA strand and the DNA strand. Other oligonucleotides may contain nucleotides containing polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may have morpholino backbone structures (U.S. Patent No. 5,034,506). Oligonucleotides may also contain groups such as reporter groups, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an antisense oligonucleotide. Antisense oligonucleotides may also have sugar mimetics.

The antisense nucleic acid molecules may be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. The antisense nucleic acid molecules of the invention or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene e.g. phosphorothioate derivatives and acridine substituted nucleotides. The antisense sequences may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced.

The antisense oligonucleotides may be introduced into tissues or cells using techniques in the art including vectors (retroviral vectors, adenoviral vectors and DNA virus vectors) or physical techniques such as microinjection. The antisense oligonucleotides may be directly administered *in vivo* or may be used to transfect cells *in vitro* which are then administered *in vivo*. In one embodiment, the antisense oligonucleotide may be delivered to macrophages and/or endothelial cells in a liposome formulation.

Peptide mimetics of podocalyxin may also be prepared as podocalyxin modulators or antagonists. Such peptides may include

competitive inhibitors, enhancers, peptide mimetics, and the like. All of these peptides as well as molecules substantially homologous, complementary or otherwise functionally or structurally equivalent to these peptides may be used for purposes of the present invention.

"Peptide mimetics" are structures which serve as substitutes for peptides in interactions between molecules (See Morgan et al (1989), Ann. Reports Med. Chem. 24:243-252 for a review). Peptide mimetics include synthetic structures which may or may not contain amino acids and/or peptide bonds but retain the structural and functional features of a peptide, or enhancer or inhibitor of the invention. Peptide mimetics also include peptoids, oligopeptoids (Simon et al (1972) Proc. Natl. Acad, Sci USA 89:9367); and peptide libraries containing peptides of a designed length representing all possible sequences of amino acids corresponding to a podocalyxin peptide of the invention.

Peptide mimetics may be designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of side chains with groups having different electronic properties, and by systematic replacement of peptide bonds with amide bond replacements. Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic. The mimetics may include isosteric amide bonds, or D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule. Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states. The mimetics can also include mimics of inhibitor peptide secondary structures. These structures can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins. Peptoids may also be used which are oligomers of N-substituted amino acids and can be used as motifs for the generation of chemically diverse libraries of novel molecules.

Peptides derived from podocalyxin isoforms may also be used to identify lead compounds for drug development. The structure of the peptides described herein can be readily determined by a number of methods such as

NMR and X-ray crystallography. A comparison of the structures of peptides similar in sequence, but differing in the biological activities they elicit in target molecules can provide information about the structure-activity relationship of the target. Information obtained from the examination of structure-activity relationships can be used to design either modified peptides, or other small molecules or lead compounds that can be tested for predicted properties as related to the target molecule. The activity of the lead compounds can be evaluated using assays similar to those described herein.

Information about structure-activity relationships may also be obtained from co-crystallization studies. In these studies, a peptide with a desired activity is crystallized in association with a target molecule, and the X-ray structure of the complex is determined. The structure can then be compared to the structure of the target molecule in its native state, and information from such a comparison may be used to design compounds expected to possess.

IV. Screening Assays

The present invention also includes screening assays for identifying agents that modulate podocalyxin and that are useful in modulating cancer cell growth. Agents that modulate include agents that stimulate podocalyxin (podocalyxin agonists) and agents that inhibit podocalyxin (podocalyxin antagonists).

In accordance with one embodiment, the invention provides a method for screening candidate compounds for their ability to modulate the activity of podocalyxin. The method comprises providing an assay system for assaying podocalyxin levels, assaying the levels in the presence or absence of the candidate or test compound and determining whether the compound has increased or decreased podocalyxin levels.

Accordingly, the present invention provides a method for identifying a compound that modulates podocalyxin comprising:

(a) incubating a test compound with podocalyxin or a nucleic acid encoding podocalyxin; and

(b) determining the effect of the compound on podocalyxin activity or expression and comparing with a control (i.e. in the absence of the test substance), wherein a change in the podocalyxin activity or expression as compared to the control indicates that the test compound modulates podocalyxin.

In one embodiment, the screening assay can be used to identify podocalyxin antagonists.

Accordingly, the present invention provides a screening assay for identifying an antagonist of podocalyxin comprising the steps of:

(a) incubating a test substance with podocalyxin; and

(b) determining whether or not the test substance inhibits podocalyxin activity, function or expression levels.

One skilled in the art will appreciate that many methods can be used in order to determine whether or not a test substance can inhibit podocalyxin and therefore inhibit cancer cell growth. Once a podocalyxin antagonist is identified in a screening assay, it may be tested in *in vitro* or *in vivo* assays to determine its effect on cancer cell growth.

In all of the above screening assays, the test compound can be any compound which one wishes to test including, but not limited to, proteins, peptides, nucleic acids (including RNA, DNA, antisense oligonucleotide, peptide nucleic acids), carbohydrates, organic compounds, small molecules, natural products, library extracts, bodily fluids and other samples that one wishes to test for modulators of podocalyxin.

The podocalyxin is generally immobilized in the above assays. Preferably, the podocalyxin is expressed on the surface of a cell, more preferably a cancer cell.

The screening methods of the invention include high-throughput screening applications. For example, a high-throughput screening assay may be used which comprises any of the methods according to the invention wherein aliquots of cells transfected with podocalyxin are exposed to a plurality of test compounds within different wells of a multi-well plate. Further, a high-throughput screening assay according to the invention involves

aliquots of transfected cells which are exposed to a plurality of candidate factors in a miniaturized assay system of any kind. Another embodiment of a high-throughput screening assay could involve exposing a transfected cell population simultaneously to a plurality of test compounds.

The method of the invention may be "miniaturized" in an assay system through any acceptable method of miniaturization, including but not limited to multi-well plates, such as 24, 48, 96 or 384-wells per plate, micro-chips or slides. The assay may be reduced in size to be conducted on a micro-chip support, advantageously involving smaller amounts of reagent and other materials. Any miniaturization of the process which is conducive to high-throughput screening is within the scope of the invention.

The invention extends to any compounds or modulators of podocalyxin identified using the screening method of the invention that are useful in treating cancer.

The invention also includes a pharmaceutical composition comprising a modulator of podocalyxin identified using the screening method of the invention in admixture with a suitable diluent or carrier. The invention further includes a method of preparing a pharmaceutical composition for use in modulating cancer cell growth comprising mixing a modulator of podocalyxin identified according to the screening assay of the invention with a suitable diluent or carrier.

The present invention also includes all business applications of the screening assay of the invention including conducting a drug discovery business. Accordingly, the present invention also provides a method of conducting a drug discovery business comprising:

(a) providing one or more assay systems for identifying a modulator of podocalyxin;

(b) conducting therapeutic profiling of modulators identified in step (a), or further analogs thereof, for efficacy and toxicity in animals; and

(c) formulating a pharmaceutical preparation including one or more modulators identified in step (b) as having an acceptable therapeutic profile.

In certain embodiments, the subject method can also include a step of establishing a distribution system for distributing the pharmaceutical preparation for sale, and may optionally include establishing a sales group for marketing the pharmaceutical preparation.

The present invention also provides a method of conducting a target discovery business comprising:

(a) providing one or more assay systems for identifying modulators of podocalyxin;

(b) (optionally) conducting therapeutic profiling of modulators identified in step (a) for efficacy and toxicity in animals; and

(c) licensing, to a third party, the rights for further drug development and/or sales for modulators identified in step (a), or analogs thereof.

V. Pharmaceutical Compositions

The present invention includes pharmaceutical compositions containing one or more modulators of podocalyxin. Accordingly, the present invention provides a pharmaceutical composition for use in modulating cancer cell growth comprising an effective amount of podocalyxin modulator in admixture with a suitable diluent or carrier.

In one embodiment, the present invention provides a pharmaceutical composition for use in treating cancer comprising an effective amount of a podocalyxin antagonist in admixture with a suitable diluent or carrier.

Such pharmaceutical compositions can be for intralesional, intravenous, topical, rectal, parenteral, local, inhalant or subcutaneous, intradermal, intramuscular, intrathecal, transperitoneal, oral, and intracerebral use. The composition can be in liquid, solid or semisolid form, for example pills, tablets, creams, gelatin capsules, capsules, suppositories, soft gelatin capsules, gels, membranes, tubelets, solutions or suspensions. The podocalyxin or ligand is preferably injected in a saline solution either intravenously, intraperitoneally or subcutaneously.

The pharmaceutical compositions of the invention can be intended for administration to humans or animals. Dosages to be administered depend on individual needs, on the desired effect and on the chosen route of administration.

The pharmaceutical compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985).

On this basis, the pharmaceutical compositions include, albeit not exclusively, the active compound or substance in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids. The pharmaceutical compositions may additionally contain other anti-cancer agents.

A pharmaceutical composition comprising the nucleic acid molecules of the invention (such as antisense oligonucleotides) may be used in gene therapy to treat cancer. Recombinant molecules comprising a nucleic acid sequence encoding podocalyxin molecule of the invention, or fragment thereof, may be directly introduced into cells or tissues *in vivo* using delivery vehicles such as retroviral vectors, adenoviral vectors and DNA virus vectors. They may also be introduced into cells *in vivo* using physical techniques such as microinjection and electroporation or chemical methods such as coprecipitation and incorporation of DNA into liposomes. Recombinant molecules may also be delivered in the form of an aerosol or by lavage. The nucleic acid molecules of the invention may also be applied extracellularly such as by direct injection into cells.

The following non-limiting example is illustrative of the present invention:

EXAMPLE 1

MATERIALS AND METHODS

Tissue Microarray Construction

A total of 270 formalin-fixed, paraffin-embedded primary invasive breast cancer tissue blocks (archival cases from Vancouver General Hospital from the period 1974-1995) that had been graded according to the Nottingham modification of the Scarth, Bloom, Richardson method (Elston and Ellis, 1991) were used to construct a tissue microarray (TMA) as described previously (Parker et al., 2002). Briefly, a tissue-arraying instrument (Beecher Instruments, Silver Springs MD) was used to create holes in a recipient block with defined array coordinates. Two 0.6 mm diameter tissue cores were taken from each case and transferred to the recipient block using a solid stylet. Three composite high-density tissue microarray blocks were designed and serial 4μm sections were then cut with a microtome and transferred to adhesive-coated slides. Normal breast and kidney tissues were used as controls.

TMA Immunohistochemistry, Scoring and Correlation Analysis

Array and control tissue sections were deparaffinized and treated for 30 min at 90°C with citrate buffer (pH 6.00) for antigen retrieval. The sections were then treated with 3% hydrogen peroxide in PBS for 30 min followed by incubation with the mouse monoclonal anti-human podocalyxin antibody 3D3 (1:80 dilution in 1% BSA in PBS; Kershaw et al., 1997a) overnight. Antibody binding was detected using the Envision detection system (Dako) and the sections were then counterstained with hematoxylin, dehydrated and mounted.

Staining of the TMA sections was scored semi-quantitatively based on the intensity of cytoplasmic staining and the proportion of cells stained: 0 - no specific staining in the tumor cells; 1 - diffuse, weak immunoreactivity or strong cytoplasmic staining reaction in <10% of the tumor cells; 2 - diffuse intermediate immunoreactivity or strong cytoplasmic staining in 10-50% of cells; 3 - strong cytoplasmic staining in >50% of the tumor cells. In the case of discrepancy between two cores from the same tumor sample,

the higher score was used. All samples were evaluated and scored independently without knowledge of the patient's outcome information.

All scores were entered into a standardized Excel spreadsheet and processed using the software TMA-deconvoluter 1.06, Cluster and TreeView programs as previously described (Liu et al., 2002). Survival analysis was performed using the Kaplan-Meier method. Paired correlation analysis to nodal status, grade, size and p53, ER, PR, and HER2 status, all of which were previously assessed on the TMA (Parker et al., 2002, Liu et al., 2002; Makretsov et al., 2003) was performed using the bivariate two-tailed Pearson test. Multivariate survival analysis was performed using the Cox proportional hazard regression model. Differences were considered significant at $p<0.05$.

Cell Culture, Transfection and Podocalyxin Localization

T47D, MCF-7 and MDA-231 human breast cancer cell lines were maintained in DMEM/F12 medium supplemented with 5% FBS (Hyclone) and insulin (5mg/ml). Endogenous podocalyxin expression was determined by Western blotting of whole cell lysates (20µg total protein) using the antibody described above for the tissue array analysis.

MCF7 cells, which expressed low levels of endogenous human podocalyxin (see Figure 2A) were transfected with a control empty pIRES-EGFP expression vector (BD biosciences) or with the same vector containing a full length mouse podocalyxin cDNA inserted into the multiple cloning site (BD Biosciences) using DMRIE-C reagent (Life Technologies/BRL). Stable transfectants were generated by continuous selection under G418 (500µg/ml; Life Technologies/BRL). Successful transfection was determined by EGFP expression which, as expected, was heterogenous given that the transfectants were uncloned pools. Podocalyxin transgene expression, (which was also heterogeneous) was determined by immunofluorescence of confluent monolayers using an antibody specific for mouse podocalyxin (Doyonnas et al., 2001). The precise subcellular localization of the mouse podocalyxin was determined by confocal microscopy after dual staining of either the adherens junction protein E-cadherin (mouse monoclonal,

Pharmingen, San Diego CA) or of the tight junction proteins occludin and ZO-1 (mouse and rat monoclonals respectively, Zymed, San Francisco CA). Here the heterogenous nature of the pooled populations was useful as it clearly demarcated consistent differences in the cell junctions of podocalyxin expressing cells.

RESULTS

Podocalyxin Expression is Weak to Negative in Normal Breast Tissue

Normal kidney sections were immunostained with anti-human podocalyxin as a positive control for antibody specificity (Kershaw DB et. al., 1997a). As expected, podocalyxin was highly expressed on glomerular podocytes cells while expression was low to negative on tubular cells (Figure 1A). This confirmed the specificity of immunocytochemical staining under the conditions used. Podocalyxin was also present in normal breast tissue but its expression was limited and it was spatially restricted. Specifically, podocalyxin was localized to the apical-most border in luminal epithelial cells (Figure 1B; arrows). In addition, podocalyxin was present on the apical face of vascular endothelial cells as has been described previously (Figure 1A, B; arrowheads, Kershaw et al.1995, McNagny et al. 1997).

Podocalyxin is Expressed by Invasive Breast Carcinoma

To determine whether podocalyxin is upregulated by neoplastic breast tissue, an array of breast tissue samples was screened using an anti-podocalyxin antibody as probe. The clinicopathological characteristics of the 270 cases that made up the tissue microarray (TMA) are shown in Table 1. Sixty-one percent (165/270) of the invasive breast carcinoma cases on the TMA exhibited no discernable podocalyxin staining and were given a designation of '0' (Figure 1C). Twenty-three percent (61/270) of the cases on the TMA exhibited weak staining in the majority of the tumor cells and they were given a designation of '1' (Figure 1D). Eleven percent (31/270) of the cases exhibited a mixture of weak and intense -membrane staining (Figure 1E).These three groups could not be distinguished from each other on the basis of clinical outcome. Specifically, Kaplan-Meier analysis of the overall

survival (data not shown) and disease free survival (Figure 2A) indicated that these three classifications were indistinguishable in terms of outcome.

Five percent (13/270) of the cases on the TMA exhibited a strong staining in the majority of the tumor cells and were originally given a designation of '3' (Figure 1F). This designation had a significantly poorer outcome compared to the other three original designations as assessed by Kaplan Meier curve analysis (Figure 2A; $p<0.02$). Therefore, this difference was statistically significant and readily observable when the 0, 1, and 2 designations were grouped and described as 'low or no podocalyxin' and compared to designation 3 described as 'high podocalyxin' (Figure 2B $p<0.02$). In addition, the high podocalyxin tumors had a mean survival time of 9.5 +/- 1.9 years, which was significantly shorter than the mean survival time of 15 +/-0.5 years for the combined low or no podocalyxin tumors. It was concluded that high level expression of podocalyxin is selective to the most metastatic tumors.

<u>High Podocalyxin Expression is an Independent Marker of Poor Outcome</u>

The same TMA that was used for podocalyxin staining has been previously stained for a number of markers that have prognostic significance for breast cancer outcome (Makretsov et al., Submitted and see www.pathology.ubc.ca/immuno). Thus, the inventors were able to perform a multi-variant Cox regression analysis in which high podocalyxin expression was compared with 6 other breast cancer-associated markers (Table 2). As expected, nodal status and HER2 overexpression were independent markers of poor outcome, which is an internal validation of the array analysis. Therefore, the fact that high podocalyxin expression on its own was associated with increased relative risk ($p<0.006$) indicates that it is an independent progonostic indicator of poor outcome. Interestingly, however, a Pearson correlation analysis of the same data indicated that high podocalyxin expression positively correlated with p53 mutations, Estrogen receptor loss, and increased tumor grade (Table 3; all p values <0.01). Thus, the data suggest that podocalyxin is an independent marker of metastatic tumors.

Ectopic Podocalyxin Expression leads to Disruption of Tight Junctions and delamination of MCF-7 breast tumor cells

Previously it has been shown that ectopic expression of podocalyxin in kidney epithelial cells (MDCK), leads to disruption of cell junctions (Takeda et al., 2000). To determine if the same is true of breast carcinoma cells the inventors first examined endogenous levels of podocalyxin in human breast tumor lines. Specifically, MCF-7 and T-47D cells, which both are capable of forming cell junctions and morphogenic structures, expressed low levels of endogenous human podocalyxin compared to the high levels of expression in the highly invasive and metastatic MDA231 cells which do not form cell junctions (Figure 3A). To test the functional significance of this expression, human MCF-7 cells were transfected with a control EGFP-expressing vector or the same vectorencoding EGFP and a full-length mouse podocalyxin. After selection drug resistance, the morphology of pooled heterogeneous populations of primary transfectants was examined. Control monolayers formed flat confluent monolayers that were undistinguishable from the parent line (data not shown). In contrast, pooled populations stably transfected with the EGFP/Podocalyxin vector contained areas where cells bulged outward from the monolayers (Figure 3B). As these cultures reached confluence they often shed podocalyxin-expressing cells into the media. Coordinate, yet heterogeneous, expression of EGFP and mouse podocalyxin was confirmed by dual green channel fluorescence and immunostaining (Figure 3B). Note also that podocalyxin was appropriately targeted to the apical membrane domain in the transfected cells (Figure 3B lower panel).

Attempts to subclone high podocalyxin expressing cells failed as these cells were constantly shed from the substratum and were difficult to maintain in suspension. The inventors therefore attempted to more fully analyze the heterogeneous pooled populations produced in the primary transfections. This allowed the effects of heterogeneous podocalyxin overexpression on cell junctions to be analyzed by dual immunostaining. Interestingly, cells expressing low to negligible levels of the podocalyxin

transgene formed normaladherens junctions with the expected basolateral expression of E-cadherin and apical expression of the tight junction protein, occludin along the lateral membranes at sites of cell-cell interaction (Figure 3C). In contrast, E-cadherin and occludin both became widely distributed on the entire surface of highly overexpressing podocalyxin expressing cells (Figure 3C). The latter cells were clearly being extruded from the monolayers as evidenced by their morphology and upward migration of their DAPI-stained nuclei. These data suggested that high levels of Podocalyxin expression can disrupt tight junction-dependent apical/basal polarity in mammary carcinoma cells. This conclusion was further supported by the finding that transepithelial resistance, which is a functional measure of tight junctions, was reduced from 497 +/- 37.2 ohms/cm2 in control-transfected MCF-7 monolayers to 210 +/- 11.9 ohms/cm2 in EGFP/Podocalyxin-transfected monolayers. Upexpression of podocalyxin in breast carcinoma cell lines leads to the disruption of cell-cell junctional complexes, mislocalization of cadherins and occludins and delamination from basement membranes, all features common to more aggressive forms of metastatic breast cancer.

DISCUSSION

The present inventors have demonstrated that abnormally high podocalyxin expression is a novel prognostic indicator of poor outcome in invasive breast carcinoma. Tissue microarrays afford investigators the opportunity to carry out a rapid and relatively thorough screening of molecules that are believed to be important in specific tissues or pathologies (Kononen et al., 1998). The power of this technology is exemplified here where only 13 of the 270 cases on our TMA had uniformally high podocalyxin expression and yet this is clearly informative with respect to prognostic outcome. The inventors are currently assembling a 3000 case invasive breast cancer TMA linked to treatment and outcome that should allow this resolving power to be increased significantly and evaluate the role of different therapies on podocalyxin status of tumors.

Locally invasive breast cancers can have markedly different treatment responses and outcomes. Thus, it is extremely difficult to predict

which patients will most benefit, or not benefit, from adjuvant therapy (Eifel et al., 2001). Genome-wide searches and large-scale expression profiling followed by cluster analysis have had some impact on this problem (Polyak et al., 2002), particularly with respect to identifying those tumors that do not progress (van't Veer et al., 2002). Despite these advances, the identification of novel independent indicators of poor outcome continues to be useful, even if they are only important in a small proportion of tumors, because they facilitate the development of new classification parameters that increase the resolving power of high throughput genomic and expression approaches. In addition, if these markers play a functional role in the biology of metastatic progression they may be rational therapeutic targets and further experimental investigations may lead to the discovery of other functionally relevant molecules in progression. This has clearly been proven to be the case with erbB2 (Nabholtz and Slamon, 2001).

The biological function of the CD34 family of sialomucins has, until recently, been quite controversial. The founding member of this family, CD34, was initially identified over 20 years ago as an hematopoietic stem cell and vascular endothelial marker and has alternatively been proposed to act as an: 1) enhancer of proliferation, 2) a blocker of differentiation, 3) bone marrow homing receptor, 4) cell adhesion molecule, and 5) a blocker of cell adhesion (Fackler et al, 1996, Krause et al. Blood, 1996, Baumhueter et al. 1993). Deletion of the CD34 gene in mice has only served to fuel this debate as these mice are relatively normal with very subtle defects in hematopoietic and vascular function. The discovery that podocalyxin and endoglycan represent additional members of the same family has made it clear that functional redundancy could be a confounding factor in any gene targeting analyses and that defects can only accurately assessed in tissues where these molecules are expressed singly (Doyonnas et al., 2001, Sassetti et al., 1998). In this regard, the most clear-cut experiments suggest that CD34-type proteins can act as either pro-adhesive or anti-adhesive molecules depending on their glycosylation status (Baumhueter 1995 and Bistrup, et al JCB 1999). Thus, CD34 and podocalyxin, expressed by high endothelial venules (HEV)

are decorated with the appropriate glycosylations to make them adhesive ligands for L-selectin expressed by circulating lymphocytes. This type of posttranslational modification is exquistly tissue-specific and the vast majority of endolthelial cells and hematopoietic cells expressing CD34 type proteins lack this modification. On all other cell types, the data suggest that these molecules serve as blockers of adhesion via their bulky, negatively-charged mucin domains, as has been demonstrated by both loss- and gain-of-function experiments (Doyonnas et al,2001 and Takeda et al. MBC 2000). The experiments described here (see below) clearly delineate an anti-adhesive role for podocalyxin and it will now be interesting to determine whether metatastic tumors lacking this molecule show increased expression of one of the other family members (CD34 or Endoglycan).

Initial functional experiments suggest that forced podocalyxin over-expression disrupts tight junctions in well-behaved MCF-7 breast carcinoma cells. Specifically, transepithelial resistance, a functional indicator of tight junction patency was significantly reduced and the spatially-restricted tight junction-associated protein occludin became very diffusely localized. Moreover, it was found that the tight junction-associated, PDZ domain-containing protein ZO-1 was mislocalized and relocalized basally in podocalyxin expressing cells (data not shown). These observations indicate that podocalyxin can function as an anti-adhesive molecule in breast cancer cells and they agree with previous findings in kidney epithelial cells where podocalyxin overexpression was shown to disrupt tight junction function and protein localization (Takeda et al., 2000) *in vitro* and podocalyxin loss was shown to lead to inappropriate tight-junction maintenance *in vivo*. In future experiments it will be interesting to determine if the potential PDZ-binding site at the extreme C-terminus of the podocalyxin cytoplasmic domain (Doyonnas et al., 2001; Takeda et al., 2001) contributes to this disruption of the tight junction. As this site also contributes to the association of podocalyxin with the actin cytoskeleton it may be involved in the cytoplasmic mislocalization of the protein itself that we observed in high expressing breast tumors (see Figure 1F).

The adherens junction protein E-cadherin is often downregulated in lobular breast carcinomas but not in the much more prevalent ductal forms of the disease. Forced expression of podocalyxin did not cause a loss of E-cadherin expression in MCF-7 cells. Instead, it altered its localization. Specifically, E-cadherin remained at the membrane but rather than being restricted to the basolateral domain the adherens junction protein was found along the entire circumference of high podocalyxin expressing cells that were being extruded from the MCF-7 monolayers. This could explain the somewhat paradoxical observation that circumferential E-cadherin localization is associated with poor outcome in grade III ductal breast carcinomas (Gillet et al., 2001). It also suggests that high podocalyxin expression may be disrupting apical-basal polarity in breast epithelial cells, which is also one function of abnormal erbB2 signaling (Brugge). A loss of polarity has been assumed to be functionally important in breast carcinoma progression, but this possibility has not yet been formally tested (Roskelley and Bissell, 2002). The inventors are currently carrying out such experiments using a 3-dimensional model of normal, polarized mammary epithelial cell morphogenesis (Roskelley et al., 2000).

Circumstantial evidence suggests that podocalyxin expression may be upregulated in a variety of neoplastic scenarios. For example podocalyxin was recently identified as the peanut agglutinin-binding tumor antigen gp200 expressed on human embryonal carcinomas. (Schopperle et al., 2002). In addition, the human podocalyxin gene (PODXL) has been assigned to chromosome 7q32-q33 (Kershaw et al., 1997), which places PODXL very close to the 7q35ter region that has been identified as a gain site by comparative genomic hybridization in ductal carcinoma in situ, infiltrating ductal carcinoma and in lymph node metastasis (Aubele et al., 2000). Thus, while it is not yet clear whether the PODXL gene is amplified in breast carcinoma, its expression may be unduly influenced by a nearby amplicon. Under anemic conditions the inventors have recently shown that Podocalyxin expression is upregulated in mouse erythroid progenitor cells (McNagny submitted unpublished obs). Therefore, podocalyxin expression may be

similarly upregulated in necrotic breast carcinomas where hypoxia-regulated genes are transcriptionally activated (Adenyinka et al., 2002). If this is indeed the case, it would have functionally important implications as tumor hypoxia helps to drive solid tumor progression generally (Knowles and Harris, 2001) and ductal carcinoma progression specifically (Bos et al., 2002; Helczynksa et al., 2003). Although a detailed dissection of the podocalyxin promoter regulatory elements has not yet been performed, it has recently been shown to be a direct transcriptional target of the Wilm's Tumor suppressor protein, WT1 (Palmer RE et al. Current Biology 2001). The role of WT1 in tumor progression is, at present, contentious. A tumor suppressive effect of this protein is supported by its loss in renal tumors and its ability to induce differentiation and cell cycle arrest of kidney and hematopoietic lineage cells. On the other hand, upregulation of WT1 expression is frequently observed in acute myeloid and lymphoid leukemias. An explanation for this apparent paradox could be the disrupted circuitry in tumor cells. For example WT1 may induce both a differentiation and cell cycle arrest program in normal cells, whereas tumor cells may have become refractory to the cell cycle arrest and only express differentiation antigens like podocalyxin.

While the present invention has been described with reference to what are presently considered to be a preferred example, it is to be understood that the invention is not limited to the disclosed example. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Table 1 Tissue Microarray Population Characteristics.

Lymph node status:	
Negative	160 (66.9%)
Positive	79 (29.3%)
Unknown	31 (11.5%)
Tumor grade:	
1	55 (20.4%)
2	148 (54.8%)
3	67 (24.8%)
Tumor Size	
<10mm	20 (7.4%)
10mm-20mm	43 (15.9%)
>20mm	72 (26.7%)
Unknown	135 (50%)
Overall Survival	
Mean	14.9 years
Median	15.0 years

Table 2 Cox Regression Multi-Variant Analysis

Marker	Degree of Freedom	Significance (p)*	Relative Risk (RR)	95% Confidence Interval for RR	
				Lower	Upper
Podocalyxin	1	0.006	7.271	1.747	30.255
P53	1	0.121	2.794	0.764	10.222
ER**	1	0.541	0.866	0.547	01.372
HER2	1	0.008	4.661	1.485	14.624
Nodes	1	0.003	3.688	1.581	08.601
Grade	2	0.257	3.088	0.798	11.946
Tumor Size	2	0.482	1.115	0.475	02.620

* Correlation is significant at the 0.05 level.
** PR gives the same result.

Table 3: Pearson Correlation Analysis Between Podocalyxin and Other Known Clinicohistopathological Markers.

Marker	Pearson Correlation	Significance	Number of Cases
Podocalyxin	1.0	-	270
p53	0.180	0.006	236
ER	-0.214	0.001	240
HER2	-0.032	0.613	258
Nodes	-0.069	0.285	239
Grade	0.191	0.002	270

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE SPECIFICATION

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WE CLAIM:

1. A method of detecting cancer in a patient comprising:

(a) obtaining a sample from the patient;

(b) determining the level of podocalyxin in the sample; and

(c) comparing the level of podocalyxin in the sample to a control sample, wherein increased levels of podocalyxin as compared to the control indicates that the patient has cancer.

2. A method according to claim 1 wherein the cancer is breast cancer.

3. A method according to claim 1 wherein the level of nucleic acid molecules encoding podocalyxin are determined in step (b).

4. A method according to claim 3 wherein the level of expression of podocalyxin mRNA is determined.

5. A method according to claim 1 wherein the level of the podocalyxin protein is determined in step (b).

6. A method according to claim 5 wherein an antibody is used to determine the levels of the podocalyxin protein.

7. A method of monitoring the progression of cancer in a patient comprising:

(a) obtaining a sample from a patient;

(b) determining the level of podocalyxin in the sample;

(c) repeating steps (a) and (b) at a later point in time and comparing the result of step (b) with the result of step (c) wherein a difference in the level of podocalyxin is indicative of the progression of the cancer in the patient.

8. A method according to claim 7 wherein the cancer is breast cancer.

9. A method according to claim 7 wherein the level of nucleic acid molecules encoding podocalyxin are determined in step (b).

10. A method according to claim 9 wherein the level of expression of podocalyxin mRNA is determined.

11. A method according to claim 7 wherein the level of the podocalyxin protein is determined in step (b).

12. A method according to claim 11 wherein an antibody is used to determine the levels of the podocalyxin protein.

13. A method of determining whether or not a cancer is metastatic in a patient comprising:

(a) obtaining a sample from the patient;

(b) detecting the level of podocalyxin in the sample; and

(c) comparing the level of podocalyxin in the sample to a control sample, wherein increased levels of podocalyxin as compared to the control indicates that the cancer is metastatic.

14. A method according to claim 13 wherein the cancer is breast cancer.

15. A method according to claim 13 wherein the level of nucleic acid molecules encoding podocalyxin are determined in step (b).

16. A method according to claim 15 wherein the level of expression of podocalyxin mRNA is determined.

17. A method according to claim 13 wherein the level of the podocalyxin protein is determined in step (b).

18. A method according to claim 17 wherein an antibody is used to determine the levels of the podocalyxin protein.

19. A kit for detecting cancer in a patient comprising (i) reagents for conducting a method according to claim 1 and (ii) instructions for its use.

20. A kit according to claim 19 wherein the reagents comprise nucleic acid primers for amplifying mRNA coding for podocalyxin in a reverse transcriptase polymerase chain reaction.

21. A kit according to claim 19 wherein the reagents comprise antibodies specific to the podocalyxin protein.

22. A kit for monitoring the progression of cancer in a patient comprising (i) reagents for conducting a method according to claim 7 and (ii) instructions for its use.

23. A kit according to claim 22 wherein the reagents comprise nucleic acid primers for amplifying mRNA coding for podocalyxin in a reverse transcriptase polymerase chain reaction.

24. A kit according to claim 22 wherein the reagents comprise antibodies specific to podocalyxin protein.

25. A kit for determining whether or not a cancer is metastatic in a patient comprising (i) reagents for conducting a method according to claim 13 and (ii) instructions for its use.

26. A kit according to claim 25 wherein the reagents comprise nucleic acid primers for amplifying mRNA coding for podocalyxin in a reverse transcriptase polymerase chain reaction.

27. A kit according to claim 25 wherein the reagents comprise antibodies specific to podocalyxin protein.

28. A method of modulating cancer cell growth by administering an effective amount of an agent that modulates podocalyxin to a cell or animal in need thereof.

29. A method of inhibiting cancer cell growth or treating cancer comprising administering an effective amount of podocalyxin antagonist to a cell or animal in need thereof.

30. A method according to claim 29 wherein the podocalyxin antagonist is an antibody that binds podocalyxin.

31. A method according to claim 29 or 30 wherein the cancer is breast cancer.

32. A method for identifying a compound that modulates podocalyxin comprising:

(a) incubating a test compound with podocalyxin or a nucleic acid encoding podocalyxin; and

(b) determining the effect of the compound on podocalyxin activity or expression and comparing with a control, wherein a change in the podocalyxin activity or expression as compared to the control indicates that the test compound modulates podocalyxin.

33. A screening assay for identifying an antagonist of podocalyxin comprising the steps of:

(a) incubating a test substance with podocalyxin; and

(b) determining whether or not the test substance inhibits podocalyxin activity, function or expression levels.

34. A pharmaceutical composition for use in modulating cancer cell growth comprising an effective amount of podocalyxin modulator in admixture with a suitable diluent or carrier.

35. A pharmaceutical composition for use in treating cancer comprising an effective amount of a podocalyxin antagonist in admixture with a suitable diluent or carrier.

ABSTRACT OF THE DISCLOSURE

Methods and kits for detecting cancer and monitoring cancer progression are described. The method involves analyzing a sample containing nucleic acids or proteins from a patient for increased expression of podocalyxin.

Figure 1

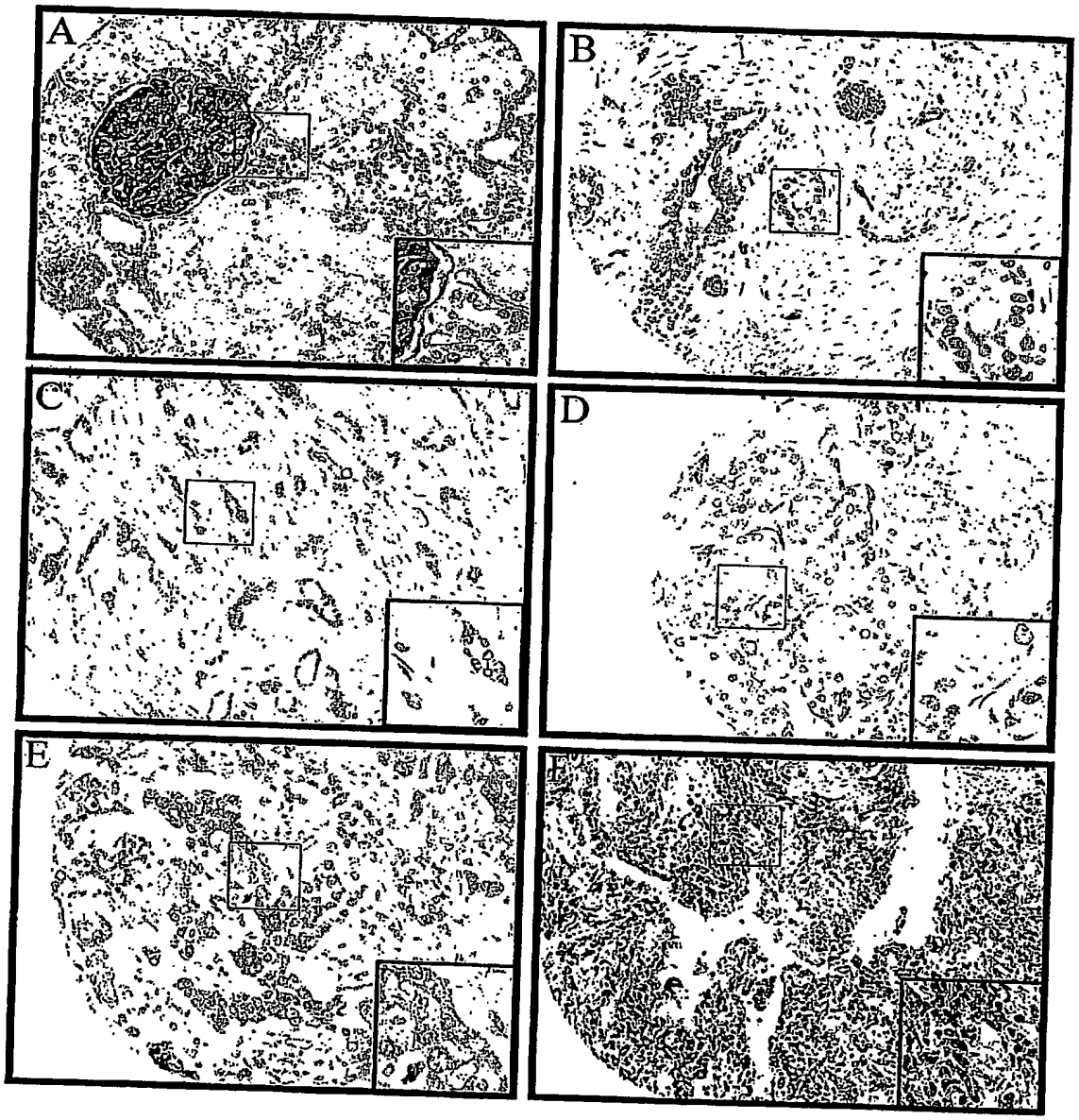
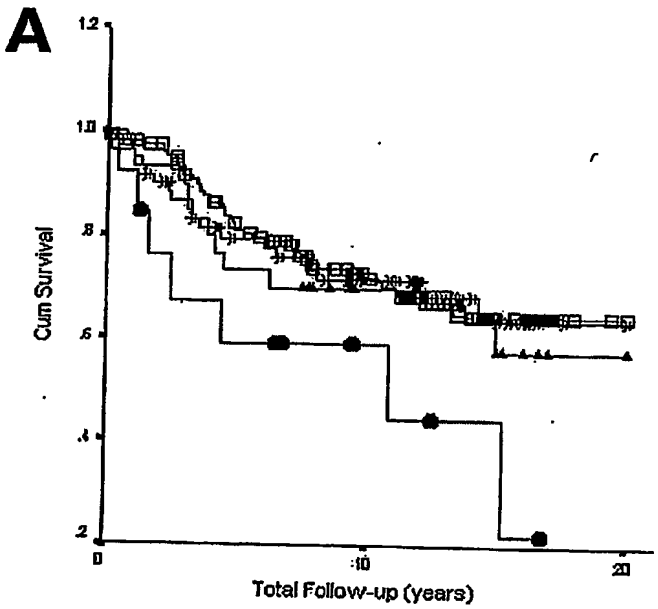


Figure 2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Cum Survival
0
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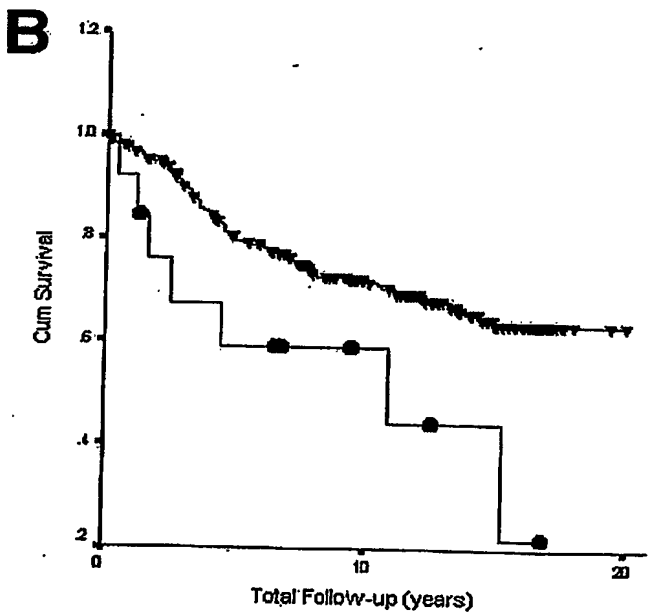
Total Follow-up (years)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Cum Survival
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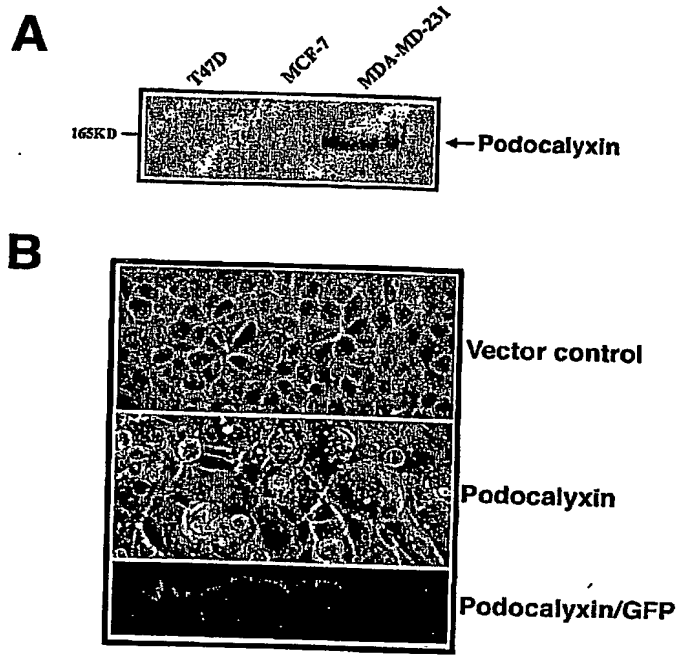
Total Follow-up (years)

Figure 3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